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Effect of *in vitro* digestion on bioactive compounds, antioxidant and antimicrobial activities of coffee (*Coffea arabica* L.) pulp aqueous extract

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ABSTRACT

Effect of *in vitro* digestion on bioactive compounds, biological activities of coffee pulp extract (CPE) against pathogens and a probiotic (*Lactobacillus acidophilus* TISTR 1338) was investigated. Total phenolic compound (TPC), chlorogenic acid (CGA), caffeine (CF), total monomeric anthocyanin (TMA), antioxidant and antimicrobial activities of the CPE were determined before and after digestion. After the digestion, the TPC, CGA and CF decreased 7.9, 31.7 and 50.0%, dry weight (dw), respectively. The antioxidant activity decreased 22.6% (DPPH) and 12.4% (FRAP). The CPE inhibited *Escherichia coli* TISTR 780 and *Staphylococcus aureus* TISTR 1466 at 150 and 200 mg/mL, respectively. Both CPE and the digested CPE had no effect on the tested probiotics. These results suggest that bioactive compounds of CPE may degrade during *in vitro* digestion, consequently the antioxidant and antimicrobial properties. Therefore, CPE could be a potential natural antimicrobial for food industry with no effect on the probiotics.

1. Introduction

The world coffee consumption has been increasing owing to its pleasant aroma and taste as well as beneficial effects on health, leading to a large amount of by-product generation. Coffee cherry pulp or coffee pulp is the main by products from the coffee processing industry contributing to approximately 45–55% of the whole coffee cherry (Murthy & Madhava, 2012). The coffee pulp is normally left naturally decomposed and used as a fertilizer in the coffee field. This natural decomposition is very slow and generates an unpleasant smell and a large amount of wastewater. The wastewater disposal problems and odor can cause environmental problems to the local community. The coffee pulp, the main by-product from coffee processing, contains valuable substances such as bioactive compounds e.g. chlorogenic acid, caffeine, cyanidins and tannins, carbohydrates (35.0–85.0% dry weight, dw), soluble fibers (30.8%, dw), proteins (5.0–11.0%, dw) and minerals (3.0–11.0%, dw), (Janissen & Huynh, 2018). Bioactive compounds presented in coffee pulp exert antioxidant properties which help to prevent cells against free radical damage and related response chronic diseases e.g. cancer (Castaldo, Narváez, Izzo, Graziani, & Ritieni, 2020). Additionally, coffee pulp has been documented to have antimicrobial activity against bacteria. According to Duangjai et al. (2016), Coffee pulp aqueous extract revealed stronger antibacterial activity on gram negative bacteria, Staphylococcus aureus, and *S. epidermidis* than gram negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*.

Human stomachs and small intestines generally contain probiotic bacteria such as *Lactobacillus* spp. to help digestion and alter bioactive compounds by the microbiota enzymes, contributing human health beneficial effects (Wojtunik-Kulesza et al., 2020). Various works have reported that the gastrointestinal digestion could affect some bioactive compounds by altering their structures, consequently their biological properties. Those effects intensity depends on various factors, such as the active compounds and their sources, extraction method, digestion condition, food matrix, and etc. (David, Danciu, Moldovan, & Filip, 2019; Rodríguez-Solana et al., 2019; Wojtunik-Kulesza et al., 2020). Several works documented on the decrease of bioactive compounds after *in vitro* gastrointestinal digestion study, for instance, chlorogenic acid in spent coffee ground between 10 and 20% (Vamanu, Gatea, & Pelinescu, 2020) and anthocyanins of wild blueberry extract 15% (Correa-Betanzo et al., 2014) compared to the undigested samples.

Recently, valorization of agro-industry by-products is an eco-friendly option that paves the way to a sustainable and economically attractive

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environmental solution. The by-products could be an alternative natural source of bioactive compounds with potential health benefits and significant market value (Castaldo et al., 2020). High value and functional food ingredients recovered from inexpensive by-products like coffee pulp could be effectively applied as functional foods, dietary supplements, fortified foods, etc. (Murthy & Madhava, 2012). Nowadays, natural antioxidant and antimicrobial agents are increasingly replacing the synthetic ones in the food industry as a result of consumer health concerns. Their biological activities could be impaired during the digestion process and might affect the probiotics in the digestive system, causing various adverse effect to the host health. Various researches have observed the antimicrobial activity of the compounds, even after the gastrointestinal digestion, against some food pathogens. For example, Lasik, Gumienna, and Nowak (2007) reported that onion and garlic extract exhibited higher antimicrobial activity in the gastric stage than the small intestine, followed by large intestine stage. This high antimicrobial activity in the gastric stage due to the synergistic effect of the low pH in the gastric digestion, while the dilution effect in the small and large intestine stage (Lasik et al., 2007).

Up until now, there have been few reports on the alteration of bioactive compounds or biological activities, particularly antioxidant and antibacterial activities of coffee pulp extract (CPE) through *in vitro* digestion model. This study was hypothesized that CPE could inhibit food pathogens, but not probiotics even after *in vitro* digestion. Therefore, this work aimed to investigate the effect of *in vitro* digestion on the bioactive compounds and the antioxidant activities. Besides, the activity of the coffee pulp extract against some pathogens (*Escherichia coli* TISTR 780*, and Staphylococcus aureus* TISTR 1466) and a probiotic bacterium (*Lactobacillus acidophilus* TISTR 1338) and change of the activity after the digestion were also investigated. This investigation would be a valuable information for possible applications of coffee by-products to the food industry or other related fields as proven by *in vitro* gastrointestinal digestion study model.

2. Material and methods

2.1. Chemicals and reagents

Folin–Ciocalteu's phenol was purchased from Merck (Germany). Gallic acid, Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), DPPH (2,2-diphenyl-1-picryhydrazyl) and TPTZ (2,4,6-tripyridy-*s*-triazine) were purchased from Sigma-Aldrich Corp. (USA). Pancreatin and pepsin were from Sigma-Aldrich (Singapore). Anhydrous sodium carbonate and butylated hydroxyl toluene (BHT) was procured from Merck (Darmstadt, Germany). Muller-Hinton Broth and plate count agar were from Difco Co. Ltd. All chemicals were of analytical grade.

2.2. Collection of samples

Arabica coffee (*Coffea Arabica* L.) pulp was kindly provided by Doi Chaang Original Co. Ltd. Chiang Rai Province, Thailand in January 2015. The pulp was stored at −40 °C until further analysis.

2.3. Microorganisms

Escherichia coli TISTR 780 (Gram negative bacteria), and *Staphylococcus aureus* TISTR 1466 (Gram positive bacteria), the cause of various outbreaks and food recalls and *Lactobacillus acidophilus* TISTR 1338, the probiotic normally found in human body, were acquired from culture collection center in Thailand called Thailand Institute of Scientific and Technological Research (TISTR).

2.4. Coffee pulp extract (CPE) preparation

The coffee pulp was homogenized with distilled water at the ratio of 1:2 (w/v) using a household blender. The mixture was filtered, then the filtrate was centrifuged (10,000 rpm) at 4 °C for 10 min. The supernatant was dried by freeze drying to obtain the CPE powder. Then the bioactive compounds (Total phenolic compound; TPC, chlorogenic acid; CGA, caffeine; CF and total monomeric anthocyanin; TMA), antioxidant activity (DPPH and FRAP assays) and antimicrobial activity by disc diffusion assay of the CPE were determined before and after *in vitro* gastrointestinal digestion.

2.5. In vitro gastrointestinal digestion

The *in vitro* gastrointestinal digestion was conducted using Faller, Fialho, and Liu (2012) with some modifications. Briefly, 1 mL of CPE at an appropriate concentration was mixed with saline solution (140 mM NaCl, 5 mM KCl and 150 μM BHT) (a final volume of 4.5 mL). The gastric digestion was initiated by adjusting the pH of the mixture to 2.0 with 0.1 M HCl and 125 μM of pepsin solution (200 mg of pepsin in 5 mL of 0.1 M HCl) was subsequently added. The gastric digested mixture was incubated in a shaker at 37 °C for 1 h. The pH of the mixture was adjusted to 6.9 with 0.1 M $NaHCO_3$ to initiate the intestinal digestion stage followed by the addition of 625 μM of pancreatin solution (37 mg of pancreatin in 18.7 mL of 0.1 M $NaHCO_3$) and incubation of the intestinal mixture was performed in a shaker at 37 °C for 2 hrs. After the designated time the pH of the collected sample was adjusted to 9.0 with 0.1 M $NaHCO_3$ and the sample was frozen at −20 °C to cease the enzyme activity. The digested sample solution, the so-called digested CPE, was thawed at ambient temperature and centrifuged (1800×g) for 10 min before further testing.

2.6. Bioactive compound determination

2.6.1. Total phenolic content (TPC)

Total phenolic content (TPC) was tested by Singleton, Orthofer, and Lamuela-Raventós (1999) method with some modifications. The diluted extract (0.5 mL) was mixed well with 2.5 mL of 10% v/v Folin-Ciocalteu's reagent and 2.0 mL Na_2CO_3 (7.5%, w/v) and mixed well. The mixture was then incubated at room temperature for 60 min before measuring the absorbance at 765 nm. The TPC was calculated as gallic acid equivalents (GAE)/100 g CPE dry weight (dw) using a gallic acid standard curve (0–100 μg/mL).

2.6.2. Chlorogenic acid (CGA) and caffeine content (CF)

Chlorogenic acid (CGA) and caffeine (CF) in coffee pulp extract and the digested fractions were determined by the method of Belay, Ture, Redi, and Asfaw (2008) and Belay and Gholap (2009), respectively. The CPE solution was mixed with dichloromethane at a ratio of 10:10 (v/v) and the mixture was vortexed for 10 min. The chlorogenic acid and the caffeine was separated using a separating funnel yielding the upper layer and the lower layer, respectively. Samples were extracted four times, each with 10 mL of dichloromethane. The absorbance of chlorogenic acid (dissolved in distilled water) was determined by spectrophotometry at wavelength of 324 nm with distilled water as a blank. The CGA of the CPE was presented as mg CGA/100 g CPE (dw). The caffeine extract (dissolved in dichloromethane) was subjected for spectrophotometry at 274 nm. Dichloromethane was applied as blank, the CF was expressed as mg CF equivalents/100 g CPE (dw).

2.6.3. Total monomeric anthocyanin (TMA)

Total monomeric anthocyanin contents (TMA) of CPE and digested fractions were evaluated by the pH-differential method (Lee, Durst, & Wrolstad, 2005). Two separated aliquots of the sample were diluted with 0.025 M KCl (pH 1) and 0.4 M CH_3COONa (pH 4.5) and subjected for spectrophotometer measurement at 520 and 700 nm, respectively. The blank was DW. The TMA molecular weight of 429.2 g/mol content and molar absorption coefficient (ε) of 26,900 L/mol, were applied for calculation. The TMA content was expressed as cyanidin-3-*O*-glycoside (C-3-G) equivalents in mg/100 g CPE (dw).

Table 1
Bioactive compounds in the CPE before and after *in vitro* gastrointestinal digestion and their reduction (%).

Bioactive compounds	*In vitro* digestion		Reduction (%)
	Before	After	
Total phenolic compound (g GAE*/100 g CPE dw)	1.13 ± 0.01[a]	1.04 ± 0.01[b]	7.9 ± 0.01
Chlorogenic acid content (g/100 g CPE dw)	1.22 ± 0.03[a]	0.83 ± 0.01[b]	31.9 ± 0.02
Caffeine content (g/100 g CPE dw)	0.16 ± 0.01[a]	0.08 ± 0.01[b]	50.0 ± 0.01
Total monomeric anthocyanin content (mg C-3-R/100 g CPE dw)	1.14 ± 0.11	ND**	

*GAE = Gallic acid equivalent, C-3-R = Cyanidin-3-glucosidide, CPE = coffee pulp extract, dw = dry weight. Each value is expressed as mean ± SD (n = 3). Means which different letters in the same row are significant different ($p \leq 0.05$). **ND, not detected.

2.7. Antioxidant activity determination

2.7.1. DPPH radical scavenging activity (DPPH)

The DPPH of the CPE was evaluated using the method previously described by Brand-Williams, Cuvelier, and Berset (1995) with some modifications. CPE and digested fractions were diluted with distilled water to an appropriate concentration. DPPH solution (60 μM in methanol, 1950 μL) was added to the diluted extract (50 μL) and mixed well. Absorbance at 517 nm was measured after leaving the reaction mixture incubated in the dark for 60 min using methanol and DW as blank and control, respectively. The DPPH was calculated from standard curve of Trolox (0–1000 μM) and presented as millimole (mmol) Trolox equivalents (TE)/100 g CPE (dw).

2.7.2. Ferric reducing antioxidant power activity (FRAP)

The FRAP of the coffee pulp extracts was performed by the method described by Benzie and Strain (1996) with modifications. The FRAP reagent (10 mM TPTZ®, 20 mM $FeCl_3$ and 300 mM sodium acetate buffer pH 3.6) at the ratio 1:1:10 (v/v/v) were freshly prepared. The diluted extract (0.2 mL) was mixed well with FRAP reagent (1.8 mL), warmed in the temperature of 50C in a water bath for 30 min. The absorbance was measured at 595 nm using DW as blank. $FeSO_4$ standard curve (0–1000 μM) was used for FRAP value calculation and expressed as mmol $FeSO_4$ equivalents/100 g CPE (dw).

2.8. Determination antimicrobial activity by disc diffusion method

Antimicrobial activity was determined by the disc diffusion method using the Clinical and Laboratory Standards Institute method (CLSL, 2006). The sterile cotton swab, previously dipped in 0.5 McFarland standard bacterial suspension, was wiped evenly onto Mueller-Hinton agar for *E. coli* TISTR 780, and *S. aureus* TISTR 1466, and onto MRS agar for *L. acidophilus* TISTR 1338. The CPE and the digested CPE were diluted to an appropriate concentration with sterile DW and 20 μL of the sample was taken to impregnate onto a 6.0 mm diameter-sterile paper disc and left to dry. The dried disc was placed onto the aforementioned microbial lawns. The inhibition zone was measured (mm) after incubation of the plate at 37 °C for 24 hrs.

2.9. Statistical analysis

For verification of the results, all experiments were performed in triplicate. SPSS version 17 was employed to perform data analyses with the confidence level of 95% ($p \leq 0.05$).

Table 2
Antioxidant activities of the CPE before and after *in vitro* gastrointestinal digestion and their reduction (%).

Antioxidant activities	*In vitro* digestion		Reduction (%)
	Before	After	
DPPH assay (mmol TE/100 g CPE dw)	3,369.59 ± 74.72[a]	2,606.32 ± 86.32[b]	22.6 ± 1.09
FRAP assay (mmol $FeSO_4$/100 g CPE dw)	17,513.04 ± 66.37[a]	15,337.83 ± 187.14[b]	12.4 ± 0.8

DPPH = DPPH radical scavenging, FRAP = Ferric reducing power, TE = Trolox, $FeSO_4$ = Ferrous sulphate, CPE = coffee pulp extract, dw = dry weight. Each value is expressed as mean ± SD (n = 3). Means which different letters in the same row are significant different ($p \leq 0.05$).

3. Results and discussion

3.1. Bioactive compounds of the CPE

The reduction of TPC, CGA, CF and TMA in the CPE after the *in vitro* digestion compared with their original content are shown in Table 1. The TPC of the CPE before the *in vitro* digestion was 1.13 g GAE/100 g CPE dw which is similar to the TPC in the coffee pulp from India (1.48 g GAE/100 g CPE dw) (Murthy & Madhava, 2012). The CGA, CF and TMA in the CPE were 1.22 g, 0.16 g and 1.14 mg C-3-G/100 g CPE dw), respectively. Similar results were reported by (Punbusayakul & Setha, 2014). After *in vitro* digestion, the TPC was slightly decreased from 1.13 to 1.04 g GAE/100 g CPE (dw) and calculated to 7.9% reduction. However, the major losses of CGA, CF and TMA were observed after the digestion. The reduction of CGA and CF were reported up to 31.9% and 50.0%, respectively, whereas TMA was not detected after the digestion.

The *in vitro* digestion has been reported to reduce TPC and could cause the disappearance of anthocyanin (Velderrain-Rodríguez et al., 2016). The decrease in TPC could be associated with the instability of phenolic compounds (caffeic acid, gallic acid, chlorogenic acid, etc.) at high pH and thus transformed to other molecules with none or less biological activity (Friedman & Jürgens, 2000). This molecular transformation occurs at the OH groups attached to the benzene ring of the phenolic compounds which structurally changes at high pH (Friedman & Jürgens, 2000). The decrease in phenolic compounds in late intestinal stage maybe because of chemical reactions that promote the oxidation, transformation and polymerization of phenolic compounds, resulting in the generation of other complex phenolic derivatives with less biological activity and poor absorbability (Altunkaya, Gökmen, & Skibsted, 2016). In addition, the loss of phenolic compounds after simulated digestion may possibly result from the interactions among the phenolic compounds and other digestive mixtures, such as buffers and electrolytes (Velderrain-Rodríguez et al., 2016). Velderrain-Rodríguez et al. (2016) also stated that the intestinal condition could reduce the phenolic compounds as a result of its instability at the intestinal alkaline pH.

Correa-Betanzo et al. (2014) showed the substantial reduction of TPC and anthocyanin as 49% and 15%, respectively after *in vitro* digestion compared to the non-digested wild blueberry extract. Thuengtung, Niwat, Tamura, and Ogawa (2018) reported changes in anthocyanins of both pigmented rice grain and slurry forms while digesting simulation. They reported the increase of anthocyanins in the gastric stage, but the decrease of anthocyanins was observed at the initial intestinal stage. They concluded that anthocyanins are susceptible to the small intestine condition, consequently low bioavailability (Thuengtung et al., 2018). Therefore, the reduction and particularly disappearance of the compounds may be due to the reaction of hydrolytic enzymes during passage through the small intestine, the effect of pH or compound transformation when they form complex with compounds in the food matrix as reported by various researchers (da Encarnação, Farrell, Ryder, Kraut, & Williamson, 2015).

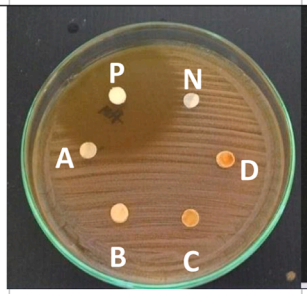
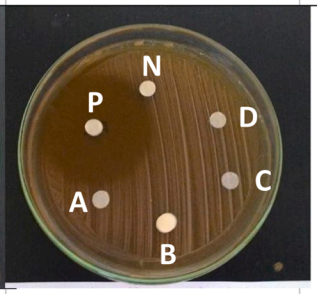
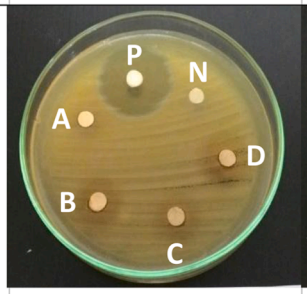
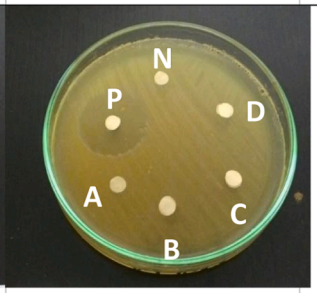
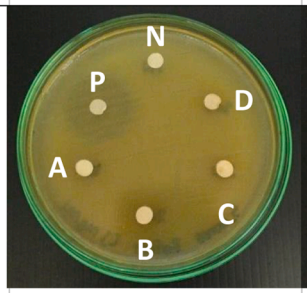
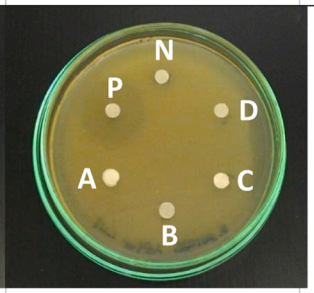
Microorganism	Before *in vitro* digestion	After *in vitro* digestion	Inhibition effect
Lactobacillus acidophilus TISTR 1338			NZ
Escherichia coli TISTR 780			NZ (200 mg/ mL opaque not completely inhibit)
Staphylococcus aureus TISTR 1466			NZ (150 mg/ mL opaque not completely inhibit)

Fig. 1. Inhibitory effect (inhibition zone, mm) of the CPE against *Lactobacillus acidophilus* TISTR 1338, *Escherichia coli* TISTR 780 and *Staphylococcus aureus* TISTR 1466 before and after *in vitro* gastrointestinal digestion. NZ = no inhibition zone, **the diameter of the filter paper disc (6 mm) is included, P = Positive controls (Penicillin for *Staphylococcus aureus* and Ampicillin for *Escherichia coli* and *Lactobacillus acidophilus*), N = Negative control (Distilled water), A = 150 mg/mL coffee pulp extract (CPE), B = 200 mg/mL CPE, C = 250 mg/mL CPE, and D = 300 mg/mL CPE.

3.2. Antioxidant activity of the CPE

The antioxidant activity of the CPE by the DPPH and FRAP assays before and after *in vitro* digestion are shown in Table 2. These results showed that even after *in vitro* digestion, the CPE still exhibited as an active antioxidant, indicating by the slightly reduction of the antioxidant activity of the CPE (22.6% reduction by DPPH and 12.4% reduction by FRAP) as shown in Table 2. The polyphenolic compounds are evidently the main compounds contributing to the antioxidant properties of fruits, vegetables as well as other plant-based materials (Wojtunik-Kulesza et al., 2020). Coffee pulp contains hydroxycinnamic acids namely chlorogenic, caffeic, and ferulic acid that act as antioxidants by donating a hydrogen atom to an oxidized molecule (Duangjai et al., 2016). Thus there is a relationship between antioxidant activities and phenolic content. Furthermore, the conditions in the digestion system such as digestive enzyme and pH exhibit an ability to generate complex antioxidants that could be quantified by DPPH and FRAP (Gião et al., 2012). Moreover, some antioxidants exhibit antioxidant network mechanisms in which the antioxidants interaction regenerates their original properties (Vilas-Boas et al., 2020).

Vilas-Boas et al. (2020) also reported that CGA which is the main compound responsible for the antioxidant property of the coffee decreased as a consequent of the *in vitro* digestion. The reduction may be due to the hydrolysis and complex formation to some compounds in the food matrix, consequently reducing their activity (Vilas-Boas et al., 2020). These results are correspondence with Castaldo et al. (2020) who revealed that the antioxidant activity of the coffee silver skin extract decreased after gastrointestinal digestion. Campos-Vega et al. (2015) stated that the antioxidant activity of the spent coffee ground increased during gastric digestion. The decreased antioxidant activity in coffee is significantly correlated to the amount of CGA as reported by Vilas-Boas et al. (2020). CGA was not stable in the alkaline pH condition in the *in vitro* digestion (Vilas-Boas et al., 2020). Friedman and Jürgens (2000) reported that most antioxidant capacities of tea beverages decreased rapidly while TPC remained relatively stable throughout the *in vitro* digestion study.

These reports suggested that the antioxidant activity of those compounds was maintained, reduced or increased depending not only on their stability in the *in vitro* digestion, but also on the properties of the derivatives that formed after the *in vitro* digestion (Castaldo et al., 2020; Velderrain-Rodríguez et al., 2016; Vilas-Boas et al., 2020). Under pH elevation upon digestion stage progress, the structural transfiguration of polyphenolic compounds takes place and causes various forms with wide-ranging chemical properties. Additionally, the bioactivities of polyphenol compounds are attributed to the arrangement of the hydroxylation patterns (–OH groups) and variations in the phenolic rings. Stronger antioxidant activity can be observed based on the position of a second –OH at the ortho (*o*-) or para (*p*-) on a phenol (Castaldo et al., 2020; Wojtunik-Kulesza et al., 2020). Thus, the study of the correlation between phenolic profiles and structure on exposed activity needs to be investigated in future studies.

3.3. Antimicrobial activity of the CPE

The inhibitory effect of the CPE before and after the *in vitro* digestion was evaluated by disc diffusion method (CLSL, 2006) and the results are summarized in Fig. 1. The main compounds in the coffee pulp extract that may contribute to its inhibitory effectiveness against the microorganism's growth include chlorogenic acid, caffeine, quinic acid, and malic acid (Duangjai et al., 2016). In addition, phenolic acids, tannin and other hydroxycinnamic acids were reported to be responsible for the antimicrobial activity of the coffee pulp extract (Duangjai et al., 2016). Phenolic compounds modify the permeability of the microorganism cell membrane and alter various intracellular functions by hydrogen bonding to enzymes. The phenolics also change the cell wall rigidity, consequently integrity losses due to a variety of interactions with the cell membrane. This phenomenon may induce irreversible cytoplasmic membrane damages and the cell content coagulation, leading to the intracellular enzyme activities cease, consequently cell death (Cushnie & Lamb, 2011).

Inhibitory effect against *E. coli* TISTR 780 and *S. aureus* TISTR 1466 indicated by opaque zone was observed at CPE concentrations of 150 and 200 mg/mL, respectively. However, there was no clear zone observed for all tested bacteria after applying with digested CPE form the *in vitro* digestion. The reduction of antimicrobial potency of the digested CPE may be due to the reduction of bioactive compounds (Table 1) as well as structural disruption by the digestive enzymes and low pH in the digestive system. In addition, both CPE and digested CPE was found to have no inhibitory effect against the *Lactobacillus* species, which is consistent with Pacheco-Ordaz et al. (2018) and Panzella et al. (2017). This effect may be due to the homeostasis mechanism against the hydrogen ion of the lactic acid bacteria and also the prebiotic agent, such as melanoidins and some phenolic compounds in the coffee pulp extract (Castaldo et al., 2020).

The evidences of the CGA and CF bioavailability after *in vitro* gastrointestinal digestion (Table 1) could be further utilized in colonic fermentation study and exploited by future researches. The improvement of probiotic bacteria such as Lactobacillus and Bifidobacterium from spent coffee ground after a simulated digestion-fermentation treatment. was reported by Panzella et al. (2017). According to Vamanu et al. (2020), the remain CGA and CF of approximately 80% after the *in vitro* gastrointestinal digestion could be a crucial value in pharmaceutical and could be applied as a substrate for human microbiota modulation. These bioactive compounds could encourage the short-chain fatty acids synthesis and enrich the naturally existing probiotic bacteria, therefore *E. coli* multiplication inhibition.

4. Conclusions

The bioactive compounds and antioxidant activity of the CPE decreased after the *in vitro* digestion. Additionally, an inhibitory effect against some pathogenic bacteria was observed only in CPE before *in vitro* digestion. However, the probiotic bacteria were not inhibited by both the non-digested and the digested CPE. These results suggest that the CPE could be potentially applied as an antimicrobial and antioxidant agent in food industry. Besides, the CPE could not affect the beneficial probiotic bacteria in the human digestive system. This investigation would be a valuable information for possible applications of coffee by-products to develop of functional foods, ingredients and other related fields in the future. Additionally, in this context, the present finding paves the way to further evaluate CPE on colonic fermentation and could apply the CPE as an inexpensive source of prebiotic in the future. However, there is a need for more study on extraction, the effect of dose concentration on bioactivity properties, confirmation of antimicrobial properties by various assays as well as types of foodborne pathogen and characterization and identification of bioactive compounds.

Ethical statement

This research did not include any human subjects or animal experiments.

CRediT authorship contribution statement

Wiriya Khochapong: Investigation, Data curation, Writing - original draft. **Sunantha Ketnawa:** Writing - review & editing. **Yukiharu Ogawa:** Resources, Supervision. **Niramol Punbusayakul:** Resources, Supervision, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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